Sinus Augmentation using BMP-2 in a Bovine Hydroxyapatite/Collagen Carrier in Dogs

Jae-Kook Cha

Department of Dentistry

The Graduate School, Yonsei University

Sinus Augmentation using BMP-2 in a Bovine Hydroxyapatite/Collagen Carrier in Dogs

Directed by Professor Seong-Ho Choi

A Doctoral Dissertation
submitted to the Department of Dentistry
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Ph.D. in Dental Science

Jae-Kook Cha

December 2015

This certifies that the Doctoral Dissertation
of Jae-Kook Cha is approved.

_____

Thesis Supervisor: Seong-Ho Choi

_____

Ui-Won Jung

_____

Jung-Seok Lee

_____

Sungtae Kim

_____

Young-Taek Kim

The Graduate School
Yonsei University
December 2015

감사의 글

본 논문이 완성되기까지 부족한 저에게 지도와 격려를 아끼지 않으신 아버지와 같은 최성호 교수님, 정의원 교수님, 이중석 교수님, 김성태 교수님, 김영택 교수님께 깊은 감사를 드립니다. 그리고 부족한 논문임에도 진심 어린 조언으로 격려해주시고 따뜻한 관심으로 지켜봐 주신 김종관 교수님, 채중규 교수님, 조규성 교수님, 김창성 교수님께 감사드립니다.

연구 내내 많은 도움을 준 치주과 수련 동기들과, 선후배님들께 모두 진심으로 감사드립니다.

마지막으로 어려움이 있을 때마다 항상 저의 버팀목이 되어주시고, 물심양면으로 도움을 주신 아버지, 어머니와 장인, 장모님께 깊은 사랑과 감사를 드립니다. 무엇보다도 아이를 돌보며 내조를 아끼지 않은 제 인생의 가장 좋은 친구이자 동반자인 아내 이영주에게 저의 온 마음을 담아 감사와 사랑을 전합니다.

아울러 학업을 핑계로 많은 시간 같이하지 못했음에도 항상 아빠에게 큰 힘을 주었던 아들 차건호에게도 고마움을 전합니다.

2015년 12월

저자 씀

Table of Contents

List of Figures

List of Tables

Abstract

Sinus Augmentation Using BMP-2 in a Bovine Hydroxyapatite/Collagen Carrier in Dogs

Jae Kook Cha, D.D.S., M.S.D.

Department of Dentistry

The Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

Objective: The objective of this study was to determine the efficacy of bone morphogenetic protein 2 (BMP-2) in a bovine hydroxyapatite/collagen (BHC) carrier to augment bone formation in a canine nasal sinus model.

Material and Methods: Eight mongrel dogs, approximately 12 months old and 30 kg in weight were used. Following preparation of bilateral sinus access windows, BHC alone (control) or loaded with *E.coli*-derived BMP-2 at 0.1 mg/mL was implanted in 4 animals, and BHC loaded with *E.coli*-derived BMP-2 at 0.5 and 1.5 mg/mL was implanted in 4 animals. The animals were euthanized at 20 weeks when block sections were obtained for micro-computed tomography and histometric analyses.

Results: Total augmented volumes did not differ significantly between groups.

Histometric analysis showed significantly enhanced bone formation for the BMP-2 groups compared with control.

Conclusion: BMP-2 in a BHC carrier, even at the low 0.1-mg/mL concentration, induces osteogenic activity, enhancing local bone formation in a canine sinus model.

KEYWORDS: bone substitutes, bone tissue engineering, bone regeneration, sinus augmentation

Sinus Augmentation Using BMP-2 in a Bovine Hydroxyapatite/Collagen Carrier in Dogs

Jae Kook Cha, D.D.S., M.S.D.

Department of Dentistry

The Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

I. Introduction

A number of studies have demonstrated extensive bone formation using bone morphogenetic protein 2 (BMP-2) with different carriers in various animal sinus augmentation models (Park, 2009). Among them, BMP-2 in absorbable collagen sponge (ACS) carrier was shown to be more effective than autogenous bone grafts and have been considered the new gold standard for this indication (Lee et al., 2013). Along with the superior effects of BMP-2/ACS in preclinical models, clinical studies have demonstrated successful results following the use of BMP-2/ACS for maxillary sinus augmentation (Boyne et al., 2005; Triplett et al., 2009).

The concentration of BMP-2 to induce the most effective bone formation is still unclear and it is influenced by various factors; e.g. delivery system, release kinetics and recipient site (Seeherman et al., 2002). *In vitro and in vivo* studies using strict dose analysis have shown an inverse correlation between bone maturation and BMP-2 dose (Park et al., 2012; Song et al., 2011; Wikesjo et al., 2008; Zara et al., 2011). However, in regards to sinus augmentation, the study of BMP-2 concentration matters relative to bone formation/maturation, and occurrence/severity of adverse events has not yet been reported. A wide range of BMP-2 concentrations have been used in preclinical sinus augmentation models (Choi et al., 2012; Gutwald et al., 2010; Hanisch et al., 1997; Lee et al., 2013). We should consider that these studies were performed in different experimental animals with various carrier systems and also that the biologic response varies between recipient sites, thus, the results of these studies could not be directly compared. Therefore, a study to determine the effects of BMP-2 depending on the concentration in sinus model is needed.

The objective of this study was to determine the efficacy of BMP-2 in a bovine hydroxyapatite/collagen (BHC) carrier to augment bone formation in the canine nasal sinus model.

II. Materials and Methods

1. Experimental Animals

Eight mongrel dogs aged about 12 months and weighing approximately 30 kg were used. All had healthy dentitions and periodontal tissues without any systemic disease. The selection and management of experimental animals and surgical procedures followed a protocol approved for this study by the Institutional Animal Care and Use Committee, College of Medicine, Yonsei University (09-021).

2. Experimental Design

E. coli derived BMP-2 was provided from the Research and Development Institute of Cowellmedi (Busan, Korea). The BMP-2 expressed by *E. coli* has been described in detail previously (Lee et al., 2010). BMP-2 was reconstituted and diluted in a buffer to obtain concentrations of 0.1, 0.5, and 1.5 mg/ml, and then 250 mg of BHC (Bio-Oss Collagen, Geistlich Pharma AG, Wolhusen, Switzerland) was loaded with 200 μl of one of the three different concentrations of BMP-2 or saline (control). The BHC blocks were a uniform volume of 9 x 9 x 8 mm in width, length and height. BMP-2 was loaded using an auto pipette in a sterilized culture dish, and after allowing 10 min for the BMP-2 to adsorb onto the surface of BHC, the blocks were

placed into the nasal sinuses. The experimental sites were divided into four groups according to the dose of BMP-2 applied (n = 4 per group):

i) Control group: BHC loaded with normal saline.

ii) BHC loaded with BMP-2 at 0.1 mg/ml (total dose = 0.02 mg).

iii) BHC loaded with BMP-2 at 0.5 mg/ml (total dose = 0.10 mg).

iv) BHC loaded with BMP-2 at 1.5 mg/ml (total dose = 0.30 mg).

Control and 0.1 mg/ml BMP-2 group were assigned in each sinus of four dogs, 0.5 and 1.5mg/ml BMP-2 groups were assigned in the other four dogs.

3. Surgical Procedure

The maxillary premolars were extracted bilaterally prior to the experimental surgery and the experimental sites were allowed to heal for 2 months. The experimental surgical procedure was performed under general anesthesia. The anesthetic procedure was described in our previous report (Oh et al., 2011).

After healing following extraction, the edentulous region was accessed using buccal incisions. A horizontal incision was made at the gingival crest from the first premolar to the third premolar, from which a vertical incision was extended apically. The full-thickness flap was elevated and the lateral bone wall was removed using an 8-mm-diameter trephine bur under sufficient saline irrigation. The position of the lateral window was determined using intraoral radiographs of the edentulous area. The membrane was carefully elevated from the floor (Fig. 1a) and lateral walls, and

then the BMP-2-loaded scaffold was applied to the created space (Fig. 1b and c). The flap was repositioned using a suture material (4-0 Monosyn; glyconate absorbable monofilament, B-Braun, Aesculap, Center Valley, PA, USA). The animals were euthanized by anesthetic drug overdose at 20 weeks.

4. Radiographic Analysis

Block sections that included BHC were dissected and fixed in 10% neutral buffered formalin for 10 days (Fig. 1d). The fixed specimens were scanned in a micro computed tomography (micro-CT; SkyScan 1072, SkyScan, Aartselaar, Belgium) at a resolution of 35 μm (100 kV, 100 μA). The scanned data were saved in DICOM format, and the experimental area was reconstructed with OnDemand 3D software (CyberMed, Seoul, Korea). In all sectioned planes, the total augmented areas were identified by color coding and traced manually by one experienced examiner using the software program. The composition of the augmented area were automatically indicated according to the gray values of the threshold, and then modified manually for fine distinction between bovine hydroxyapatite (BH) and new bone (NB) in all sectioned planes (Fig. 1e). The gray values of threshold were standardized and they ranged from 145 to 225 for BH and from 95 to 145 for NB. The overall dimensional topography of experimentally grafted sinus cavities were visualized with the aid of three-dimensionally (3D) reconstructed images. The total augmented volume and

volume of BH (mm^3) were calculated by integration of the data from all tomographic images.

5. Histologic Analysis

After rinsing the specimens in sterile water, the sections were decalcified in 5% formic acid, dehydrated in a graded ethanol series, and embedded in paraffin. Serial sections were cut at a thickness of 5 μm in an apicocoronal vertical plane. The two most central sections of each grafted site were selected and stained with hematoxylin-eosin and Masson's trichrome. Histologic analysis was performed using a stereomicroscope (MZFLIII, Leica, Wetzlar, Germany) and light microscope (BX-50, Olympus Optical, Tokyo, Japan).

6. Histomorphometric Analysis

Micrographs were taken at a magnification of ×12.5 and assembled to enable visualization of the entire sinus. After examination with a conventional light microscope, histomorphometric measurements were made using a PC-based image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The histomorphometric analysis was performed by one experienced, masked examiner (J.S.L.). To minimize intraexaminer errors, several randomly selected samples were measured twice with a 1-week interval. The intraexaminer reproducibility was evaluated using the concordance correlation coefficient, which was 0.93-0.98

(Barnhart et al., 2007; Lin, 1989). Each photomicrograph was horizontally aligned and imaginary horizontal line was established according to the base of nasal sinus floor for linear measurement. The following measurements were made as primary outcome variables (Fig. 1f):

i) Total augmented area (mm^2) and height (mm): the experimentally augmented area surrounded by the floor of nasal sinus and Schneiderian membrane, including NB, residual BH particles, and FCT. Augmented height was defined as the distance from the floor of the nasal sinus to the highest point of the total augmented area.

ii) NB height proportion (%NBH; %): the proportion of the distance from the floor of the nasal sinus to the highest point of NB within the sinus (NBH) in relation to the total augmented height.

iii) Composition of the augmented area (%): the proportions of NB (%NB), residual particles (%BH), and FCT (%FCT) relative to the total augmented area.

The following additional measurements were made as secondary outcome variables, to evaluate the healing around the residual particles and the homogeneity of bone regeneration:

i) Proportions of BH particles surrounded by NB (%BHB): the proportion of the number of BH particles surrounded by NB relative to the total number of BH particles in the augmented area.

ii) Separated fractions of NB in the central and peripheral portions of the

augmented area (%; Fig. 1f): %NB in selected central and peripheral areas, respectively. The central-most vertical and horizontal area, and the horizontally central/most-coronal area were selected for this measurement.

7. Statistics

Group means (±SD) were calculated. Statistical analyses were performed separately at the intra- and inter-subject levels. Wilcoxon signed-rank test was used for the comparison between control and 0.1 mg/ml BMP-2 group, and 0.5 and 1.5 mg/ml groups. Mann-Whitney U test used to compare groups which were allocated in different animals; control versus 0.5 and 1.5 mg/ml groups, and 0.1 mg/ml group versus 0.5 and 1.5 mg/ml groups. The level of statistical significance was set at $p < 0.05$.

III. RESULTS

1. Clinical Findings

Surgical wound healing was uneventful during the experimental period. Nasal bleeding occurred immediately after the surgical procedure in several dogs, but it stopped within an hour. No complications – including membrane perforation, wound dehiscence, severe swelling, and bleeding – were observed.

2. Radiographic Analysis

The 3D reconstructed images revealed the entire shape of the augmented area (Fig. 2). In the control group, the BHC had almost maintained its original rectangular block shape over the sinus floor. However, the BH had become spread out and flattened on the sinus floor in the BMP-2-treated groups.

NB and BH could be observed in the cross-sectional images (Fig. 2). In the control group, the BH particles were still evident at the floor of the sinus and only a small amount of NB was observed inside the BHC. In the BMP-2-treated groups, the BH particles appeared to have disassembled and were scattered over the sinus floor. With a BMP-2 dose of 0.1 mg/ml, a bone bridge had formed within the BHC block so that the BH and the NB had become commingled. In the groups with BMP-2 at 0.5 and 1.5 mg/ml, the amount of BH was significantly reduced and abundant NB was

observed, homogeneous with the original sinus floor. The volume of BH in the BMP-2 treated groups tended to be lower than that in the control group, however the differences between groups were not statistically significant on intra/inter subject level tests (control group, 26.25 ± 15.01 mm^3; 0.1 mg/ml BMP-2 group, 14.50 ± 10.32 mm^3; 0.5 mg/ml BMP-2 group, 12.52 ± 16.42 mm^3; 1.5 mg/ml BMP-2 group, 11.21 ± 5.25 mm^3). Also, the total augmented volumes did not differ statistically between the groups (control group, 253.99 ± 41.00 mm^3; 0.1 mg/ml BMP-2 group, 346.61 ± 102.75 mm^3; 0.5 mg/ml BMP-2 group, 246.93 ± 95.97 mm^3; 1.5 mg/ml BMP-2 group, 317.50 ± 95.78 mm^3).

3. Histologic Findings

The nasal sinus cavity was surrounded by respiratory mucosa and a thin layer of cortical bone. The Schneiderian membrane was intact, with no sign of inflammation in all groups.

In the control group, most of the BH particles were encapsulated by dense fibroblastic cells with smooth borders in the whole augmented area, and only a small amount of NB could be detected in the original sinus floor. In the central portion of the augmented area in particular, the NB was barely visible and multinucleated osteoclast-like cells were absent (Fig. 3a, b and c).

In contrast, in the BMP-2 treated groups, a trabecular pattern of NB was observed in direct contact along the Schneiderian membrane. NB was also seen at the tented

area lateral to the BHC. In the NB induced by BMP-2, concentric layers of bone tissue were observed around Haversian canals and well-defined lamellar bone, exhibiting characteristics of mature bone. The osteoclast-like cells were absent and the osteoclastic activity was not observed around the BH particles with smooth and intact borders (Fig. 3d, e and f, Fig. 4). Interestingly, with 0.5 mg/ml BMP-2 the BH particles tightly attached to the NB had penetrated the alveolar bone beneath the original sinus floor such that the original sinus floor was barely distinguishable from the NB (Fig. 4a, b and c); moreover, with 1.5 mg/ml BMP-2, a large amount of completely remodeled NB was observed throughout the entire sinus cavity, and the BH particles were barely visible (Fig. 4d, e and f).

4. Histometric Analysis

The composition of the augmented area is summarized in Fig. 5a and Table 1. Both the area of NB (mm^2) and %NB were significantly larger in all of the BMP-2-treated groups than in the untreated control. Furthermore, the bone formation observed with BMP-2 was significantly larger than the control group not only in the central portion of augmented area close to the basal bone of the sinus, but also in the peripheral portion of augmented area near the Schneiderian membrane (Table 2).

%BHB and %NBH differed significantly between the control and 0.1 mg/ml BMP-2 group or 0.5 and 1.5 mg/ml BMP-2 groups. The values in the BMP-2-treated groups tended to increase with concentration, but the differences between the groups were

not statistically significant at all intra/inter subject level tests (Fig. 5b; %NBH: control group, 52.3 ± 27.1%; 0.1 mg/ml BMP-2 group , 97.8 ± 4.3%; 0.5 mg/ml BMP-2 group, 98.8 ± 2.3%; 1.5 mg/ml BMP-2 group, 100%; %BHB: control group, 29.2 ± 24.3%; 0.1 mg/ml BMP-2 group , 91.4 ± 8.2%; 0.5 mg/ml BMP-2 group, 97.9 ± 2.6%; 1.5 mg/ml BMP-2 group, 97.7 ± 4.6%).

Table 1. Composition of the Total Augmented Area. (mean ± standard deviation)

Group	NB (mm^2)	BH (mm^2)	FCT (mm^2)	AA (mm^2)
Control	7.04 ± 2 .43	8.29 ± 1.29	17.75 ± 1.49	33.09 ± 3.04
BMP-2 (0.1 mg/ml)	18.50 ± 1.1*	4.36 ± 0.25*	10.51 ± 2.09*	33.36 ± 3.04
BMP-2 (0.5 mg/ml)	22.05 ± 1.91*	2.01 ± 1.02*,†	9.88 ± 2.49*	33.94 ± 5.01
BMP-2 (1.5 mg/ml)	24.26 ± 5.90*	1.52 ± 0.22*,†	10.88 ± 3.09*	36.65 ± 4.18

NB, newly formed bone; BH, bovine hydroxyapatite; FCT, fibrovascular connective tissue; AA, total augmented area; BMP-2, bone morphogenetic protein 2.

* Significantly different from control group ($p < 0.05$).

† Significantly different from BMP-2 at 0.1 mg/ml ($p < 0.05$).

Table 2. Percentage of NB between the peripheral and central area of the sinus cavity. (mean ± standard deviation)

Group	Ce (%)	Pe (%)
Control	5.18 ± 0.95	10.03 ± 1.00
BMP-2 (0.1 mg/ml)	44.30 ± 5.58*	45.11 ± 7.02*
BMP-2 (0.5 mg/ml)	38.67 ± 13.80*	42.70 ± 5.79*
BMP-2 (1.5 mg/ml)	42.29 ± 19.63*	44.58 ± 7.99*

Ce, central-most vertical and horizontal areas of the augmented graft; Pe, horizontally central/most-coronal area of the augmented graft.

* Significantly different from control group ($P < 0.05$).

IV. DISCUSSION

The present study evaluated osteoinductive activities of BMP-2 in BHC using the dog nasal sinus experimental model. Osteogenic activity of BMP-2 was observed even at the lowest dose (0.1 mg/ml, corresponding to 0.02 mg of BMP-2), and this value was 15 times lower than the approved concentration for human use (1.5mg/ml with ACS), nevertheless induced twice the amount of bone regeneration compared to the control.

Previous studies have demonstrated that low quality of bone is attributable to the formation of adipose tissue when higher concentrations of BMP-2 are applied (Park et al., 2012; Song et al., 2011). However, our findings refute this since we obtained good-quality bone with limited adipogenic differentiation at all experimental sites including the ones with low and high concentrations of BMP-2. Improved bone qualities were shown even at the peripheral portion of augmented areas distant from osteogenic sources in all experimental sites, while new bone formation was barely observed in this area of the control group. This is concurrent with Choi et al. reporting that osteoinductive potential of the Schneiderian membrane is activated at the early stage of healing with BMP-2 (Choi et al., 2013). Despite the controversy of whether the Schneiderian membrane contains the osteogenic source, the authors suggested that newly formed bone from the Schneiderian membrane at the peripheral area could protect the augmented space from the volume shrinkage by remodeling process.

All experimental sites showed comparable formation of newly formed bone, regardless of the concentration of BMP-2. This indicates that increased dose of BMP-2 beyond a certain threshold would not improve bone regeneration in sinus augmentation, and the threshold concentration would be smaller than the present experimental range of BMP-2 concentration (0.1-1.5 mg/ml in 200 μl) in dog nasal sinus model. The sinus augmentation model is a contained defect surrounded by the sinus floor and the Schneiderian membrane; thus its healing process could be accelerated even with the low BMP-2 concentration. And also characteristics of the carrying system could have influenced the present result. BMP-2 may not have been fully adsorbed onto the surface of BHC during preparation, and the BMP-2 might not be biologically available along the concentration gradient. The adsorption and release profiles of BMP-2/BHC have not been verified yet, thus this feature should be fully investigated in future studies.

Interestingly, it was observed that local high dose of BMP-2 (0.5mg/ml) induced remodeling of the sinus floor and beyond (1.5mg/ml). In the process of new bone formation, a previous study has reported that the BMP-2 dose-dependently stimulated osteoclastic bone resorption as well as acted as a mediator of the osteoblast-osteoclast interaction (Kanatani et al., 1995). The scattered residual biomaterials surrounded by newly formed bone in the present results may be a product of this vigorous remodeling process enhanced by BMP-2. While the cortical portion of the original sinus floor had been resorbed away in the early healing process, BHC would have scattered and sequential new bone formation would have occurred around these

particles. Even though the collagen matrix in BHC could enhance the clinical manageability, it could be suggested that the structural integrity of BHC might be insufficient to support the maintenance of space during the healing process by BMP-2.

In the same vein, the present results demonstrated a tendency of decrease in the proportion of remaining BH at sites that received BMP-2; histological analysis revealed significant differences, but volumetric analysis on the reconstructed micro CT images did not. This could be explained by excessive swelling in the early healing phase of experimental sites and increased bone formation by BMP-2, which could have migrated the grafted biomaterial to the lateral aspect of the augmented area. BH has been considered as a non-resorbable biomaterial in craniofacial fields (Hallman et al., 2001; Hallman and Thor, 2008; Schlegel and Donath, 1998). The previous results showed that the BH particles remained similar size to the original BH particles even in 7- and 10-year biopsy samples (Mordenfeld et al., 2010; Orsini et al., 2007).

This study incorporated the nasal sinus model of the dog, which is anatomically adjacent to the maxillary premolars and has a similar bone structure to the human maxillary sinus which contains alveolar bone and the lateral wall (Aerssens et al., 1998). Histologically, it has similar compositions to the human Schneiderian membrane as the dog sinus membrane is comprised of pseudostratified ciliated columnar epithelium which is a respiratory epithelium (Lee et al., 2007). Additionally, the intraoral surgical approach in the dog nasal sinus model has a high relevance to the human clinical procedure (Wetzel et al., 1995). On the other hand, as the dog nasal sinus is connected to the nose, it is subjected to more direct transmission of

positive respiratory pressure than the human (Haas et al., 1998). Hence the present results may conservatively be interpreted for clinical application in human.

The present study used two separate statistical analyses; dependent test for comparing groups within one animal, and independent test for groups between animals. It was caused by the limitation in the number of sinuses of one subject animal; therefore, these results should be interpreted conservatively. However, the present study focused on whether the significantly reduced concentration (0.1mg/ml) of BMP-2 could affect bone healing in sinus augmentation, and the results clearly demonstrated extensive new bone formation in comparison to the control. These results were comparable to that of the conventional concentrations (0.5 and 1.5mg/ml) of BMP-2. Therefore, in continuation of the current focus of minimizing the dosage of BMP for bone regeneration, the findings of our study indicate the necessity to perform further studies with concentrations of BMP-2 lower than 0.1 mg/ml.

Within the limitations of this study, it can be concluded that BMP-2 in a BHC carrier, even at the low 0.1-mg/mL concentration, induces osteogenic activity, enhancing local bone formation in the canine sinus model.

REFERENCES

Aerssens J, Boonen S, Lowet G, Dequeker J: Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. *Endocrinology* 139(2): 663-670, 1998.

Barnhart HX, Lokhnygina Y, Kosinski AS, Haber M: Comparison of concordance correlation coefficient and coefficient of individual agreement in assessing agreement. *J Biopharm Stat* 17(4): 721-738, 2007.

Boyne PJ, Lilly LC, Marx RE, Moy PK, Nevins M, Spagnoli DB, et al.: De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 63(12): 1693-1707, 2005.

Choi Y, Lee JS, Kim YJ, Kim MS, Choi SH, Cho KS, et al.: Recombinant human bone morphogenetic protein-2 stimulates the osteogenic potential of the schneiderian membrane: a histometric analysis in rabbits. *Tissue Eng Part A* 19(17-18): 1994-2004, 2013.

Choi Y, Yun JH, Kim CS, Choi SH, Chai JK, Jung UW: Sinus augmentation using absorbable collagen sponge loaded with Escherichia coli-expressed recombinant human bone morphogenetic protein 2 in a standardized rabbit sinus model: a radiographic and histologic analysis. *Clin Oral Implants Res* 23(6): 682-689, 2012.

Gutwald R, Haberstroh J, Stricker A, Ruther E, Otto F, Xavier SP, et al.: Influence of rhBMP-2 on bone formation and osseointegration in different implant systems after sinus-floor elevation. An in vivo study on sheep. *J Craniomaxillofac Surg* 38(8): 571-579, 2010.

Haas R, Mailath G, Dortbudak O, Watzek G: Bovine hydroxyapatite for maxillary sinus

augmentation: analysis of interfacial bond strength of dental implants using pull-out tests. *Clin Oral Implants Res* 9(2): 117-122, 1998.

Hallman M, Lundgren S, Sennerby L: Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clin Implant Dent Relat Res* 3(2): 87-96, 2001.

Hallman M, Thor A: Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. *Periodontol 2000* 47: 172-192, 2008.

Hanisch O, Tatakis DN, Rohrer MD, Wohrle PS, Wozney JM, Wikesjo UM: Bone formation and osseointegration stimulated by rhBMP-2 following subantral augmentation procedures in nonhuman primates. *Int J Oral Maxillofac Implants* 12(6): 785-792, 1997.

Kanatani M, Sugimoto T, Kaji H, Kobayashi T, Nishiyama K, Fukase M, et al.: Stimulatory effect of bone morphogenetic protein-2 on osteoclast-like cell formation and bone-resorbing activity. *J Bone Miner Res* 10(11): 1681-1690, 1995.

Lee HJ, Choi BH, Jung JH, Zhu SJ, Lee SH, Huh JY, et al.: Maxillary sinus floor augmentation using autogenous bone grafts and platelet-enriched fibrin glue with simultaneous implant placement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 103(3): 329-333, 2007.

Lee J, Susin C, Rodriguez NA, de Stefano J, Prasad HS, Buxton AN, et al.: Sinus augmentation using rhBMP-2/ACS in a mini-pig model: relative efficacy of autogenous fresh particulate iliac bone grafts. *Clin Oral Implants Res* 24(5): 497-504, 2013.

Lee JH, Kim CS, Choi KH, Jung UW, Yun JH, Choi SH, et al.: The induction of bone formation in rat calvarial defects and subcutaneous tissues by recombinant human BMP-2, produced in Escherichia coli. *Biomaterials* 31(13): 3512-3519, 2010.

Lin LI: A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45(1): 255-268, 1989.

Mordenfeld A, Hallman M, Johansson CB, Albrektsson T: Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clin Oral Implants Res* 21(9): 961-970, 2010.

Oh KC, Cha JK, Kim CS, Choi SH, Chai JK, Jung UW: The influence of perforating the autogenous block bone and the recipient bed in dogs. Part I: a radiographic analysis. *Clin Oral Implants Res* 22(11): 1298-1302, 2011.

Orsini G, Scarano A, Degidi M, Caputi S, Iezzi G, Piattelli A: Histological and ultrastructural evaluation of bone around Bio-Oss particles in sinus augmentation. *Oral Dis* 13(6): 586-593, 2007.

Park JB: Use of bone morphogenetic proteins in sinus augmentation procedure. *J Craniofac Surg* 20(5): 1501-1503, 2009.

Park JC, Kim JC, Kim BK, Cho KS, Im GI, Kim BS, et al.: Dose- and time-dependent effects of recombinant human bone morphogenetic protein-2 on the osteogenic and adipogenic potentials of alveolar bone-derived stromal cells. *J Periodontal Res*, 2012.

Schlegel AK, Donath K: BIO-OSS--a resorbable bone substitute? *J Long Term Eff Med Implants* 8(3-4): 201-209, 1998.

Seeherman H, Wozney J, Li R: Bone morphogenetic protein delivery systems. *Spine (Phila Pa 1976)* 27(16 Suppl 1): S16-23, 2002.

Song DS, Park JC, Jung IH, Choi SH, Cho KS, Kim CK, et al.: Enhanced adipogenic differentiation and reduced collagen synthesis induced by human periodontal ligament stem cells might underlie the negative effect of recombinant human bone morphogenetic

protein-2 on periodontal regeneration. *J Periodontal Res* 46(2): 193-203, 2011.

Triplett RG, Nevins M, Marx RE, Spagnoli DB, Oates TW, Moy PK, et al.: Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 67(9): 1947-1960, 2009.

Wetzel AC, Stich H, Caffesse RG: Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clin Oral Implants Res* 6(3): 155-163, 1995.

Wikesjo UM, Qahash M, Polimeni G, Susin C, Shanaman RH, Rohrer MD, et al.: Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations. *J Clin Periodontol* 35(11): 1001-1010, 2008.

Zara JN, Siu RK, Zhang X, Shen J, Ngo R, Lee M, et al.: High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. *Tissue Eng Part A* 17(9-10): 1389-1399, 2011.

Figure Legends

Figure 1. (a) A trephine bur (outer diameter = 8.0 mm) was used to produce a lateral wall osteotomy, and the Schneiderian membrane (SM) was lifted with the aid of a periosteal elevator. (b) Bovine hydroxyapatite/collagen (BHC) was inserted beneath the membrane. (c) Postoperative periapical radiographic image; BHC (arrows). (d) Inner surface image of the lateral wall after decalcification in 5% formic acid. (e) Coronally sectioned image of the augmented area identified and indicated with color-coding using the software program. (f) Schematic drawing of the histometric analysis (AB, alveolar bone; NB, newly formed bone; BH, bovine hydroxyapatite; FCT, fibrovascular connective tissue; AH, total augmented height; NBH, distance from the floor of the nasal sinus to the highest point of NB in AH; Ce, central-most vertical and horizontal area; Pe, horizontally central/most-coronal area).

Figure 2. Representative 3D reconstructed and coronally sectioned micro-computed tomography images of nasal sinuses. (a, b) Control group; (c, d) with 0.1 mg/ml bone morphogenetic protein 2 (BMP-2); (e, f) with 0.5 mg/ml BMP-2; (g, h) with 1.5 mg/ml BMP-2.

Figure 3. Histologic photomicrographs from the control group (a, b and c), and the 0.1 mg/ml BMP-2-treated group (d, e and f) (stained with Masson's trichrome). (a) Low-magnification image of the entire sinus from the control group (arrows show a

small amount of NB from the original sinus floor; scale bar = 1 mm). (b) Highly magnified view of the SM (scale bar = 100 μm). (c) Highly magnified view of the peripheral part of the augmented area (scale bar = 250 μm). (d) Low-magnification image of entire sinus from the 0.1 mg/ml BMP-2 treated group. A bone bridge was formed along the SM in direct contact with it (arrowheads; scale bar = 1 mm). (e, f) Highly magnified polarized photomicrographs of the SM, and the central part of the augmented area (scale bar = 250 μm).

Figure 4. Histologic photomicrographs from the 0.5 mg/ml BMP-2-treated group (a, b and c), and the 1.5 mg/ml BMP-2-treated group (d, e and f) (stained with Masson's trichrome). (a) Low-magnification image of the entire sinus from the 0.5mg/ml BMP-2 treated group (scale bar = 1 mm). (b, c) Highly magnified polarized photomicrographs of the SM, and the central part of the augmented area (scale bar = 250 μm). (d) Low-magnification image of the entire sinus from the 1.5 mg/ml BMP-2-treated group (scale bar = 1 mm). (e) Highly magnified polarized photomicrograph of the SM (scale bar = 100 μm). (f) Highly magnified polarized photomicrograph of the central part of the augmented area (HC, Haversian canal; scale bar = 250 μm).

Figure 5. (a) Composition of the total augmented area. (b) Proportion of the number of BH particles surrounded by NB (%; asterisks indicate significant difference from the control group, $p < 0.05$).

Figures

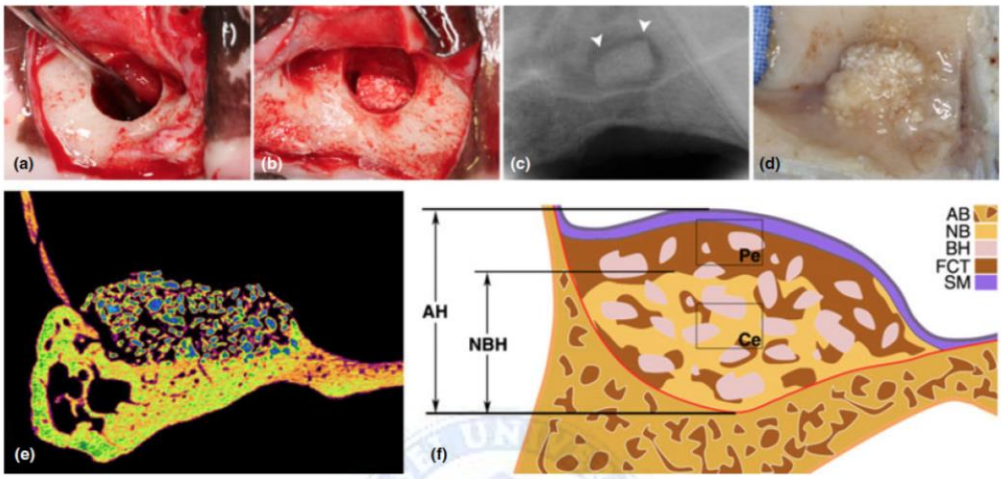

Figure 1.

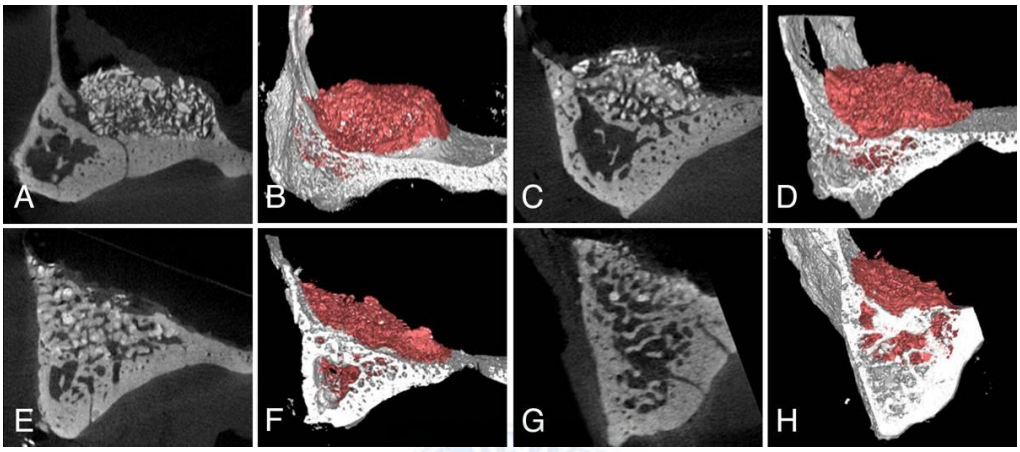

Figure 2.

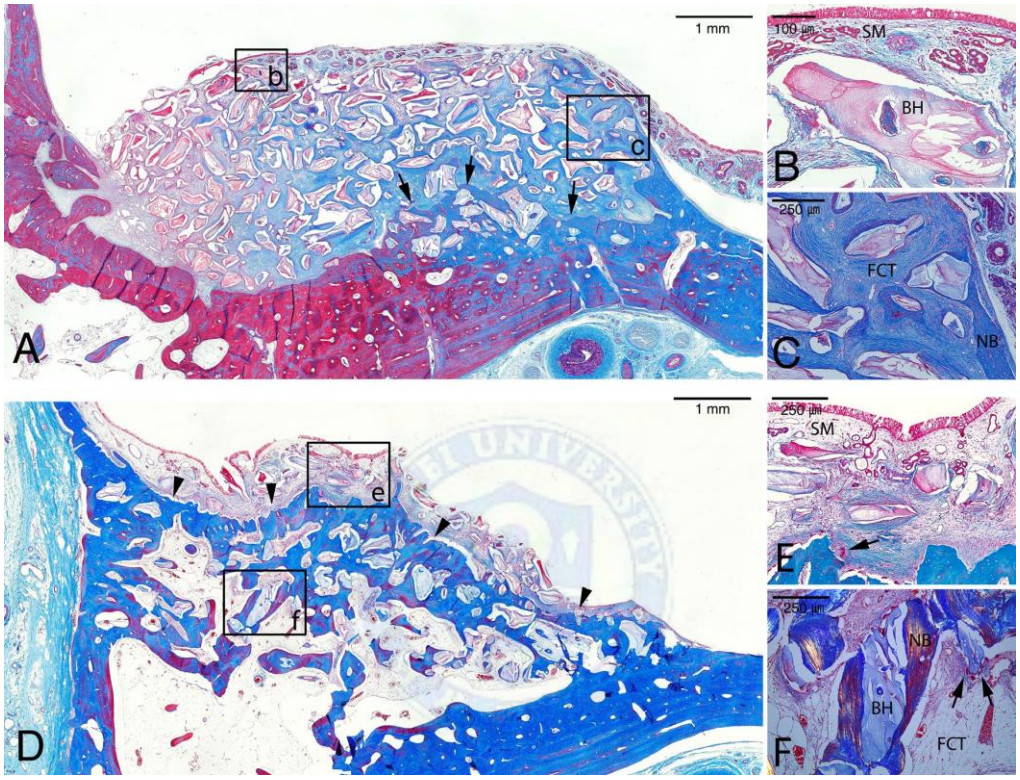
1 mm
b
c
A
100 µm
SM
BH
B
250 µm
FCT
NB
C
1 mm
e
f
D
250 µm
SM
E
250 µm
NB
BH
FCT
F

Figure 3.

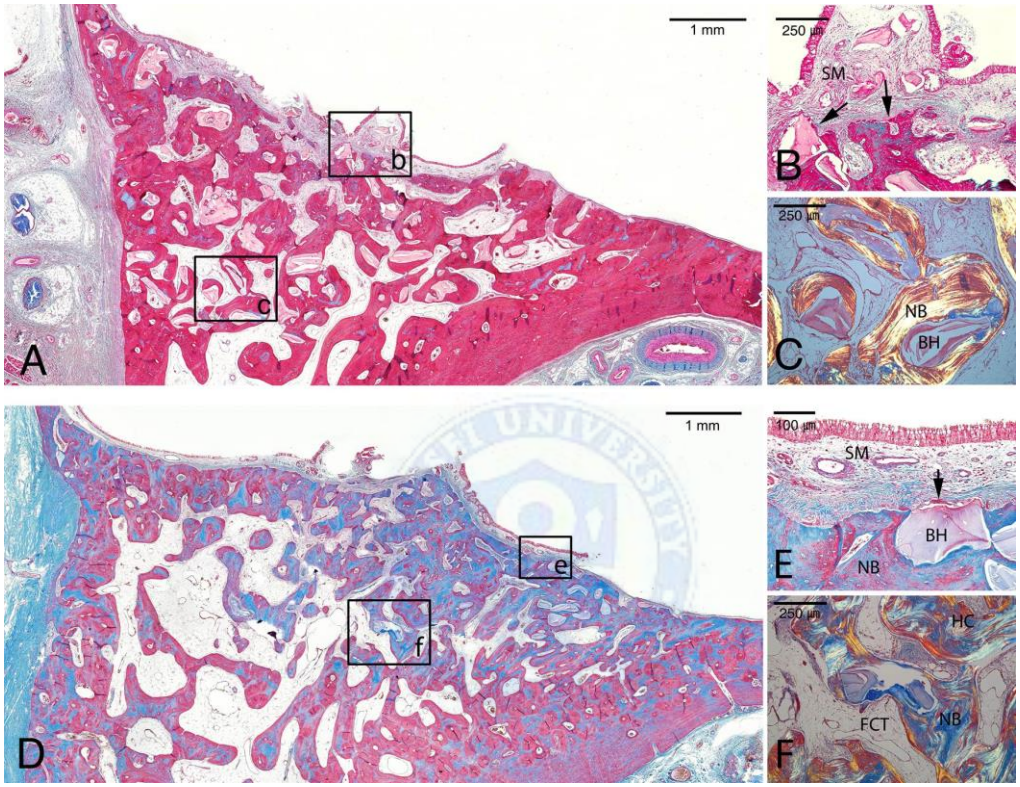
1 mm
250 µm
SM
B
b
c
250 µm
NB
BH
C
A
1 mm
100 µm
SM
BH
E
NB
e
f
250 µm
HC
FCT
NB
F
D

Figure 4.

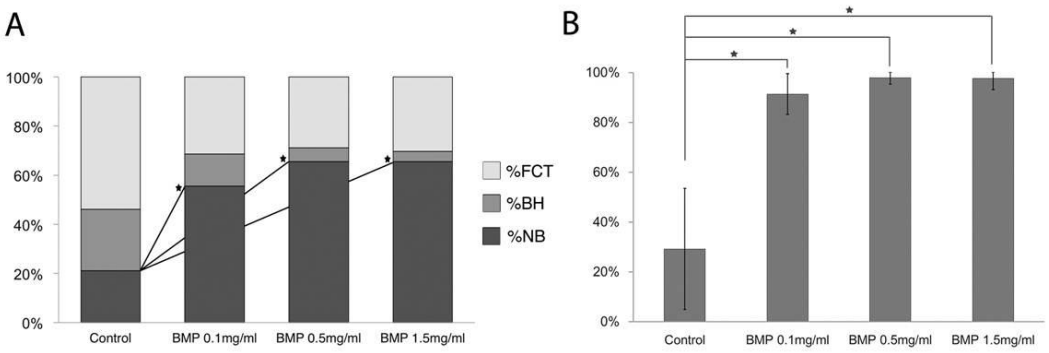
A
100%
80%
60%
40%
20%
0%
Control
BMP 0.1mg/ml
BMP 0.5mg/ml
BMP 1.5mg/ml
%FCT
%BH
%NB
B
100%
80%
60%
40%
20%
0%
Control
BMP 0.1mg/ml
BMP 0.5mg/ml
BMP 1.5mg/ml

Figure 5.

국문요약

상악동 거상술 시 탈단백우골/콜라겐 전달체를 사용한 제 2형 골형성 유도 단백질의 농도에 따른 골 형성

< 지도교수 최성호 >
연세대학교 대학원 치의학과
차 재 국

목적: 상악동에서 제 2형 골형성 유도 단백질(BMP-2)의 골 형성 효과는 여러 선행 연구를 통해 증명되었지만, 아직까지 최적의 농도는 정립되지 않았다. 상악동 거상술은 치과 술식 중 다량의 골이식재가 사용되는 술식으로, 과량의 BMP-2의 사용에 따른 부작용이 쉽게 발생할 수 있으므로 골 형성 효과를 유지하며 부작용을 최소화할 수 있는 이상적인 농도를 정립하는 과정이 필요하다. 따라서 이 연구의 목적은 개 상악동에서 탈단백우골/콜라겐 (BHC) 전달체를 사용한 BMP-2의 농도에 따른 골 형성 효과를 평가하여 최적의 농도를 결정하는 것이다.

재료 및 방법: 8마리의 잡견 양측에서 상악동 거상술을 시행하였다. 4마리 개의 한쪽 상악동에는 생리식염수를 첨가한 BHC (대조군)를, 그리고 다른 한쪽 상악동에는 0.1 mg/ml 농도의 BMP-2를 첨가한 BHC를 이식하

였다. 나머지 4마리의 개의 상악동에는 각각 0.5, 1.5 mg/ml 농도의 BMP-2를 첨가한 BHC를 이식하였다. 20주의 치유기간 후, 방사선학적 분석 및 조직 계측학적 분석을 시행하였다.

결과: 방사선학적 분석 결과 총 증대된 부피는 실험군과 대조군 간 유의한 차이를 보이지 않았다. 조직계측학적 분석에 따르면 BMP-2를 첨가한 군들에서 대조군에 비해 유의하게 증가된 신생골의 면적과 높이를 보였다. BMP-2의 농도가 증가할수록 신생골의 양은 증가하는 경향을 보였으나, 통계적으로 유의한 차이는 없었다. 상악동 내 부위에 따른 골 형성 효과를 분석한 결과 BMP 처치군은 상악동 중앙부와 주변부에서 모두 균일한 골 형성이 있었지만, 대조군의 상악동 중앙부에서는 신생골이 거의 관찰되지 않았다.

결론: 개 상악동에서 모든 BMP-2 처치군은 BHC를 전달체로 사용하여 대조군에 비해 현저하게 향상된 골 형성 효과를 보였다. 최소 농도인 0.1 mg/ml의 BMP-2도 고농도 BMP-2에 비해 유의한 차이 없는 골 형성 효과를 보였다. 따라서 개 상악동에서 BMP-2의 최적의 농도는 0.1 mg/ml 이하일 것으로 사료되며 이에 대한 추가적인 연구가 필요하다.

핵심되는 말: 골재생, 골대체제, 골조직공학, 상악동 증대술